

Tropical Ecology 53(2): 235-240, 2012
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www.tropecol.com

ISSN 0564-3295

Differences in soil moisture, nutrients and the microbial community between forests on the upper Pacific and Caribbean slopes at Monteverde, Cordillera de Tilaran: implications for responses to climate change

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Abstract: A study was conducted to identify soil ecosystem metrics for use in monitoring soil ecosystem health in tropical montane cloud forest habitats, and to establish a baseline of soil community characteristics. This work was performed in six unique forested habitats on the Caribbean and Pacific slopes of the Monteverde Reserve in Costa Rica. Comparisons were made of the soil moisture, nitrogen fixation activity, microbial biomass, fungal and bacterial abundance and diversity, and the abundance of key functional genes laccase (for lignin degradation by basidiomycete fungi) and nifH (for bacterial N-fixation). Differences were found in these metrics between habitats, which were correlated with soil moisture. The results of this work show that these metrics can be used as part of a program to monitor the soil ecosystems for early indicators of shifts in conditions in response to environmental change within this part of a fragile ecosystem.

Resumen: Se realizó un estudio para identificar las métricas del ecosistema edáfico que pueden usarse en el monitoreo de la salud de este ecosistema en hábitats de bosque nublado montano y para establecer información de línea base de las características de la comunidad del suelo. Este trabajo se llevó a cabo en hábitats forestales únicos en las laderas del Caribe y del Pacífico de la Reserva Monteverde, Costa Rica. Se hicieron comparaciones de la humedad del suelo, la actividad de fijación de nitrógeno, la biomasa microbiana, la abundancia y la diversidad de hongos y bacterias, y la abundancia de los genes funcionales clave de la lacasa (para la degradación de la lignina por hongos basidiomicetos) y nifH (para la fijación bacteriana de N). Se encontraron diferencias para estas métricas entre hábitats, las cuales estuvieron correlacionadas con la humedad del suelo. Los resultados de este trabajo muestran que estas métricas pueden usarse en programas de monitoreo de ecosistemas edáficos como indicadores tempranos de modificaciones en las condiciones como respuesta al cambio ambiental en este componente de un ecosistema frágil.

Resumo: Este estudo foi conduzido para identificar as métricas do ecossistema do solo para uso na monitorização da saúde do ecossistema em habitats florestais tropicais montanos de nevoeiros, e estabelecer uma linha básica quanto às características da comunidade do solo. Este trabalho foi efectuado em seis habitats florestados únicos nas vertentes das Caraíbas e do

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Pacífico na Reserva Monteverde na Costa Rica. Foram efetuadas comparações na humidade do solo, abundância fúngica e bacteriana e diversidade, bem como a abundância de genes funcionais chave de lacase (para a degradação da lenhina pelo basidiomicetas) e *nifH* (para as bactérias de fixação de N). Encontraram-se diferenças nestas métricas entre habitats, os quais estavam correlacionadas com a humidade do solo. Os resultados deste trabalho mostraram que estas métricas podem ser utilizadas como parte de um programa para monitorizar os ecossistemas do solo como indicadores precoces de mudanças nas suas condições em resposta à mudança climática no seio desta parte de um ecossistema frágil.

Key words: Climate change, microbial biomass, microbial community structure, monteverde, montane forest, soil moisture.

Tropical montane cloud forests require both rain and frequent immersion in clouds for their maintenance. However, the cloud forests of Monteverde, Costa Rica, are currently threatened by documented increases in mist-free periods (Lawton *et al.* 2001; Pounds & Puschendorf 2004; Ray *et al.* 2006). Modeling studies there also have provided significant reason to believe that orographic cloud base height is increasing, that frequency of forest immersion in cloud is decreasing, and that the area immersed in clouds for significant times is decreasing (Lawton *et al.* 2001; Ray *et al.* 2006). In response to this concern, the first study was conducted in these forests to compare soil moisture, nitrogen (N) fixation activity, microbial biomass, fungal and bacterial abundance and diversity, and the abundance of key functional genes laccase (for lignin degradation by basidiomycete fungi) and *nifH* (for bacterial N-fixation). This study is part of a larger project to monitor these forests for impacts of decreasing precipitation (Salazar *et al.* 2009). As such, the goals were to identify metrics for use in monitoring soil ecosystem health in these habitats over time, and to begin establishing a baseline of soil community characteristics.

Six naturally occurring and undisturbed montane cloud forest habitats, representing various Hartshorn (1983) life zones, were identified in the Monteverde Reserve, from various life zones, three on the Caribbean slope (El Valle, Aleman, Eladios) and three on the Pacific (Brillantes, Pantanoso, Alondra sites) slope. The georeferences, altitude and Hartshorn (1983) life zones are provided in Table 1. In each habitat, a single 50 x 50 m plot was established, divided into four equal subplots, and, following removal of the upper layer of soil debris, 16 randomly located 10 cm deep and 2 cm

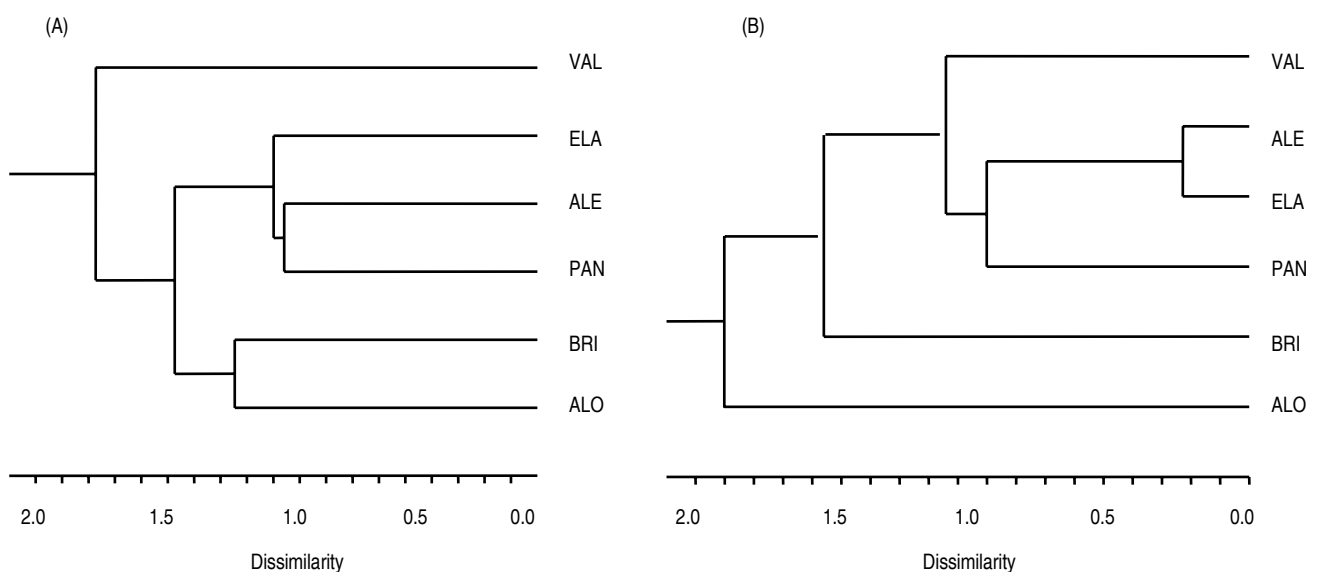
diameter soil cores were collected, pooled by subplot, and the soil sieved while field moist. The soil was composed of highly decomposed materials, and all soil cores contained only organic layer material. All data were adjusted per dry weight of the soil and bulk density, and results are presented per volume of soil. Each of the four subplot soil samples per forest type were analyzed for levels of microbial biomass C (Cmic), total mineral nitrogen (TMN), the PCR-based relative percent contribution (RPC) of bacterial rRNA (primers 27f/1492r), fungal rRNA (primers EF4/Fung5), nitrogen fixation gene (primers *nifHf*/*nifHr*), and laccase gene (primers *lacIf*/*lacII*), and the restriction fragment length polymorphism (RFLP)-based relative diversity and abundance of the bacterial and fungal community as described by Eaton *et al.* (2011).

All parameter means were analyzed for differences by 2-tailed t-test and mean effect size (*d*) analysis. To account for the small sample sizes, a weight-of-evidence approach was used in which mean differences with *P* values < 0.15, or 0.15 to < 0.25 and *d* values > 0.9 were considered to represent meaningful differences between two mean values. The RFLP data were used to determine the Shannon-Weiner diversity index (*H'* by habitat type), and for cluster analysis using a similarity matrix and Ward's minimum variance method of measuring Euclidean distances in NCSS software. Metrics most influenced or influenced by Cmic and soil moisture were identified as critical correlations for application in future monitoring and model development if correlation coefficient *r* values (at *P* = 0.1) were ≥ 0.2 or ≤ -0.2 , considered medium level correlation coefficients.

The habitats with the greatest soil moisture on both the Pacific and Caribbean slopes (Brillantes and El Valle, respectively) had the most fungal-

Table 1. Summary of the sample sites, georeferences, altitude, and Hartshorn (1983) life zones.

	Latitude	Longitude	Slope	Altitude (m)	Hartshorn Life Zone
Eladios	N10°18'26.415"	W084°43'09.909"	Caribbean	904	Premontane Rainforest
Aleman	N10°18'14.657"	W084°44'10.143"	Caribbean	912	Premontane Rainforest
El Valle	N10°19'28.531"	W084°46'07.224"	Caribbean	1563	Lower Montane Rainforest
Alondra	N10°17'56.437"	W084°47'44.653"	Pacific	1509	Lower Montane Wet Forest
Pantanoso	N10°18'19.025"	W084°47'00.082"	Pacific	1625	Lower Montane Rainforest
Brillantes	N10°17'22.251"	W084°47'02.467"	Pacific	1499	Lower Montane Rainforest

**Fig. 1.** Dendrograms comparing the relatedness of the RFLP-based diversity of bacterial and fungal community rRNA in soil collected from Pacific slope (ALO, BRI, PAN) and Caribbean slope forests (ALE, ELA, VAL). (A) Bacterial rRNA diversity, (B) Fungal rRNA diversity.

dominated soils, with greater fungal to bacterial rRNA ratios, greater amount of laccase and (generally) fungal rRNA genes, the lowest amount of bacterial rRNA gene, with less bacterial and greater fungal rRNA diversity. These habitats also had the lowest levels of total mineral N and greatest amount of *nifH* gene, which, along with the generally greater biomass, suggests that more active nitrogen fixation and incorporation of mineral N into the biomass is occurring in these forests (Tables 2 & 3). The diversity of both the bacterial and fungal rRNA of the Brillantes and Alondra soils from the Pacific slope were closely related, as were the Eladios and Aleman soils of the Caribbean slope. Both types of rRNA from the Pantanoso (Pacific slope) and El Valle (Caribbean) soils were more

closely related to those from the Caribbean slope soils (Fig. 1A & 1B). Interestingly, the Brillantes and Alondra soils also had significantly greater Cmic values than the other four soils, suggesting that the diversity and structure of the microbial populations might differ in correlation with C cycle dynamics. As well, the RFLP data showed that there were clearly unique fungal and bacterial population components in the more moist soils (Fig. 1A & 1B). When examined along with the RPC, N and biomass data, this suggests the possibility that selection is occurring for N-fixing bacteria and basidiomycete fungi (and lignin degradation) in the more moist soils of forests from both slopes.

Soil moisture influences microbial biomass (Arunachalam *et al.* 1997; Eaton 2001; Eaton *et al.*

Table 2. The mean value, standard deviation (SD), *P*-value from t-test, and mean effect size (*d*) are presented for soil moisture (as % moisture), total mineral nitrogen (TMN as $\mu\text{g g}^{-1}$), microbial biomass carbon (Cmic as mg C g^{-1}), and the PCR-based relative percent contribution (RPC) of bacterial rRNA (RPC bact), fungal rRNA (RPC fung), ratio of fungal to bacterial rRNA (Fung:bact), N- fixation gene (RPC nifH), and laccase gene (RPC lac) from Monteverde soil samples collected from different forests on the from Pacific slope (Brillantes, Alondra, Pantanos) and Caribbean slope forests (Valle, Eladidos, Aleman).

	Mean	SD	Mean	SD	Mean Difference	<i>P</i> -value	<i>d</i> -value
	Brillantes		Alondra				
% moisture	73.25	4.00	69.30	3.11	3.95	0.170	1.10
TMN	16.71	3.97	38.12	10.76	-21.42	0.010	2.64
Cmic	4.19	0.499	3.91	0.63	0.28	0.513	0.49
RPC bact	0.37	0.087	0.42	0.15	-0.05	0.555	0.44
RPC fung	0.46	0.022	0.37	0.09	0.08	0.108	1.34
Fung:bact	2.65	0.712	1.90	1.36	0.75	0.368	0.70
RPC nif	0.35	0.065	0.15	0.06	0.19	0.006	2.95
RPC lac	1.17	0.087	1.06	0.11	0.10	0.189	1.05
	Brillantes		Pantanos				
% moisture	73.30	4.00	70.80	1.34	2.50	0.281	0.84
TMN	16.71	1.83	28.39	6.62	-11.68	0.014	2.40
Cmic	4.19	0.23	3.40	0.55	0.78	0.039	1.85
RPC bact	0.37	0.04	0.33	0.07	0.04	0.385	0.66
RPC fung	0.46	0.02	0.35	0.02	0.10	0.000	5.43
Fung:bact	2.65	0.328	1.97	0.40	0.68	0.039	1.86
RPC nif	0.35	0.03	0.18	0.06	0.41	0.000	9.19
RPC lac	1.17	0.04	0.92	0.04	0.24	0.000	6.33
	Valle		Eladidos				
% moisture	65.60	1.28	62.80	0.73	2.80	0.009	2.69
TMN	22.80	2.79	29.30	1.69	-6.50	0.007	2.82
Cmic	2.78	0.11	2.85	0.17	-0.07	0.539	0.46
RPC bact	0.21	0.05	0.50	0.09	-0.29	0.001	4.12
RPC fung	0.41	0.06	0.34	0.06	0.07	0.174	1.09
Fung:bact	3.91	1.21	1.46	1.41	2.46	0.038	1.87
RPC nif	0.30	0.02	0.19	0.11	0.11	0.091	1.42
RPC lac	1.21	0.07	1.03	0.1926	0.1788	0.132	1.23
	Valle		Aleman				
% moisture	65.60	1.28	60.70	3.24	4.90	0.031	1.99
TMN	22.80	2.79	26.21	3.88	-3.41	0.203	1.01
Cmic	2.78	0.11	2.47	0.94	0.31	0.542	0.46
RPC bact	0.21	0.05	0.41	0.12	-0.21	0.020	2.23
RPC fung	0.41	0.06	0.37	0.12	0.04	0.573	0.42
Fung:bact	3.91	1.21	1.79	1.76	2.13	0.094	1.40
RPC nif	0.30	0.02	0.26	0.08	0.04	0.322	0.76
RPC lac	1.21	0.07	0.99	0.30	0.21	0.222	0.96

Table 3. The mass and diversity index (H') of the bacterial rDNA and fungal rDNA bands following RFLP analysis of DNA products from PCR amplification of soil DNA collected from Pacific slope (ALO, BRI, PAN) and Caribbean slope forests (ALE, ELA, VAL) in the Monteverde cloud forest reserve. The soil DNA was amplified by PCR methods using the universal bacterial and fungal rRNA primers described in the text.

	Bacterial rRNA PCR Products		Fungal rRNA PCR Products	
	Mass (ng) of DNA Bands	H' value	Mass of DNA Bands	H' value
BRI	519	1.47	90	1.38
ALO	369	1.62	92	1.25
PAN	309	1.54	90	1.31
VAL	453	1.16	104	1.35
ELA	241	1.46	61	1.18
ALM	160	1.66	85	1.28

2011; Schwendenmann & Veldkamp 2006), which is also influenced by a wide variety of nutrient and microbial community components (Anderson 2003; Schulze 2004) in establishing healthy-functioning soils (Doran 2002). Thus, the metrics that most influence or are influenced by soil moisture and Cmic were considered as target indicators of "soil quality" in this study. The soil moisture was positively correlated (at $P = 0.1$) with Cmic ($r = 0.52$), RPC of fungal rRNA ($r = 0.21$), fungal to bacterial rRNA ratio (0.22), laccase (0.27) and nifH genes ($r = 0.26$), and negatively correlated with the TMN levels ($r = -0.20$) and RPC of bacterial rRNA ($r = -0.24$). The Cmic was positively correlated with soil moisture ($r = 0.52$), RPC of fungal rRNA ($r = 0.21$), fungal to bacterial rRNA ratio (0.22), laccase (0.27) and nifH genes ($r = 0.26$), and negatively correlated with the TMN levels ($r = -0.20$) and RPC of bacterial rRNA ($r = -0.24$). Thus, it appears that these metrics are good indicators of differences in soil ecosystem condition.

Lower concentrations of mineralized N are associated with increased nifH gene activity (Schulze 2004), microbial biomass development (Eaton 2001; Eaton *et al.* 2011; Schwendenmann & Veldkamp 2006), and increased bacterial decomposition which triggers bottom-up processes leading to increases in fungal abundance and diversity (Currie 1999). Ele-

vated soil moisture levels increase the release of organic matter from woody debris, stimulating an increase in microbial activity, nutrient utilization, and microbial biomass development, thus lowering the pools of inorganic N (Arunachalam *et al.* 1997; Eaton 2001; Eaton *et al.* 2011; Schulze 2004; Schwendenmann & Veldkamp 2006). These factors along with lower soil oxygen levels that occur with increased soil moisture positively regulate the nifH gene activity, which is also associated with biomass development (White *et al.* 2007). Conversely, lower levels of soil moisture decrease N cycle activities and microbial biomass, and result in increased concentrations of pools of inorganic N (Eaton 2001; Eaton *et al.* 2011; Ewing *et al.* 2007), which can inhibit both bacterial N-fixation and lignin degradation by basidiomycetes, thus decreasing the microbial biomass and sequestration of organic C into the soil biota (Ewing *et al.* 2007; De Boer *et al.* 2005). The data from the current study suggest that these relationships have important implications for soil ecosystem quality should moisture levels decrease in the Monteverde forests.

This was the first study to demonstrate that measurable differences existed in the soil nutrients, microbial community, and microbial biomass in the different habitats, that were correlated with soil moisture. Due to the concern of the observed decreasing amounts of precipitation in the Monteverde cloud forest region (Pounds *et al.* 1999), the metrics suggested by this work should be used as part of a program to monitor the soil ecosystems early indicators of shifts in conditions in response to environmental change within this part of a fragile ecosystem.

Acknowledgements

This project was supported by the National Science Foundation grants DBI-0452328 and was conducted under the Costa Rican Government Permit #063-2008-SINAC. The authors especially wish to acknowledge and thank all the Tropical Science Center staff, Brenda Campbell, Emily Giles, and Robert S. J. Eaton for their incredible effort made in collecting soils samples in the field under the most difficult of circumstances.

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(Received on 09.11.2009 and accepted after revisions, on 15.07.2011)